Hiroshima Univ.

Fluorescence Microscopy-Based Method for Selective Detection of Asbestos

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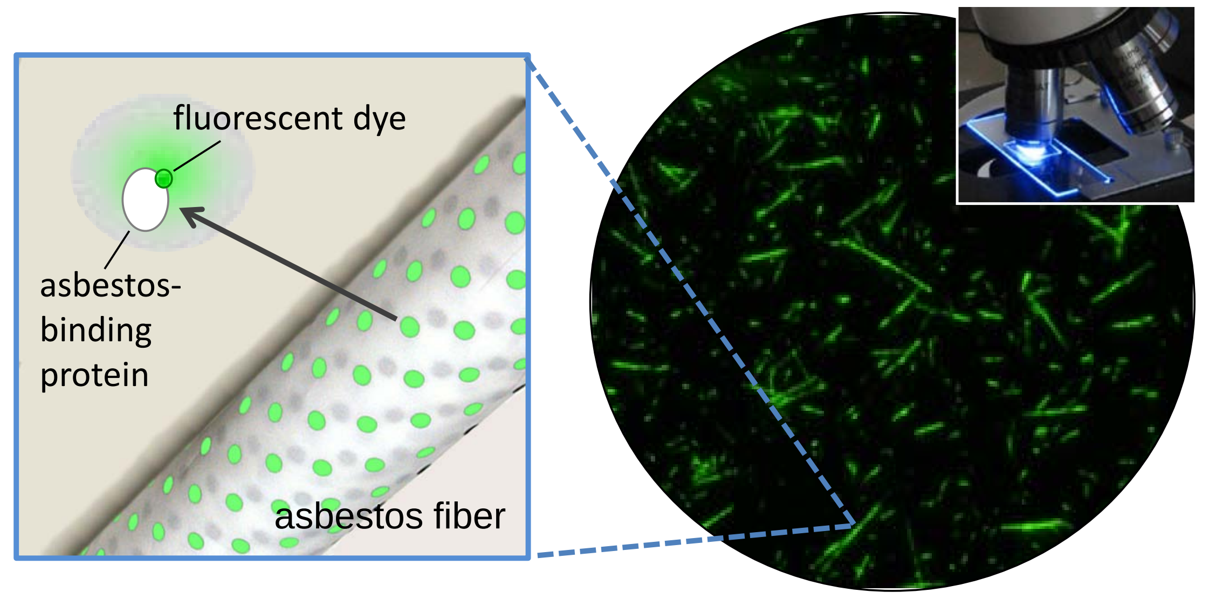
Abstract

The most commonly used method for asbestos detection in air samples relies on phase contrast microscopy (PCM). While simple and cheap, PCM has a number of limitations. It cannot detect asbestos fibers thinner than about 0.25 µm and is not able to distinguish asbestos fibers from other natural or man-made fibers of similar dimensions. Electron microscopy-based methods (EM) are more sensitive but also expensive, and require much more time for sample preparation and analysis.

Recently, we have developed a fluorescence microscopy (FM)-based method for selective and highly sensitive detection of asbestos. This method relies on staining of asbestos fibers collected on filter membrane using **a fluorescently labeled protein probe** that selectively binds to asbestos fibers (see Figure to the right). The fibers can be observed and counted using fluorescence microscope (see the pictures to the right).



1. Asbestos binding proteins

Asbestos-binding proteins DksA and GatZ have been discovered by screening a large number of bacterial proteins from *Escherichia coli* for binding to chrysotile and amosite. **DksA** bound to chrysotile (**K_d=3.5nM**, see Fig. A to the right), but neither amosite nor crocidolite. **GatZ** bound to all kinds of amphibole asbestos including amosite and crocidolite (**K_d's =22.1nM** for amosite, see Fig. B to the right).

Fig.A: chrysotile

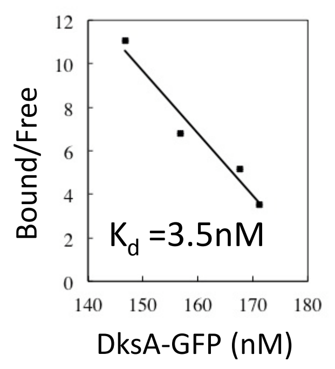
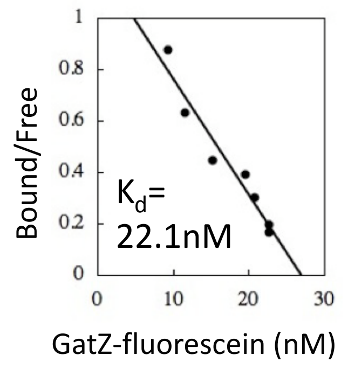
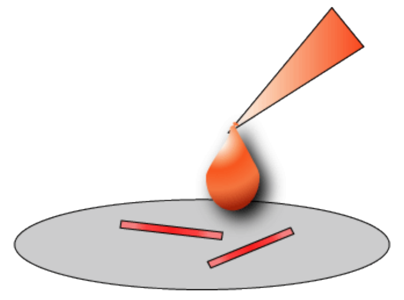


Fig.B: Amosite



3. Asbestos detection kit & Automated fiber counting

Asbestos detection kit



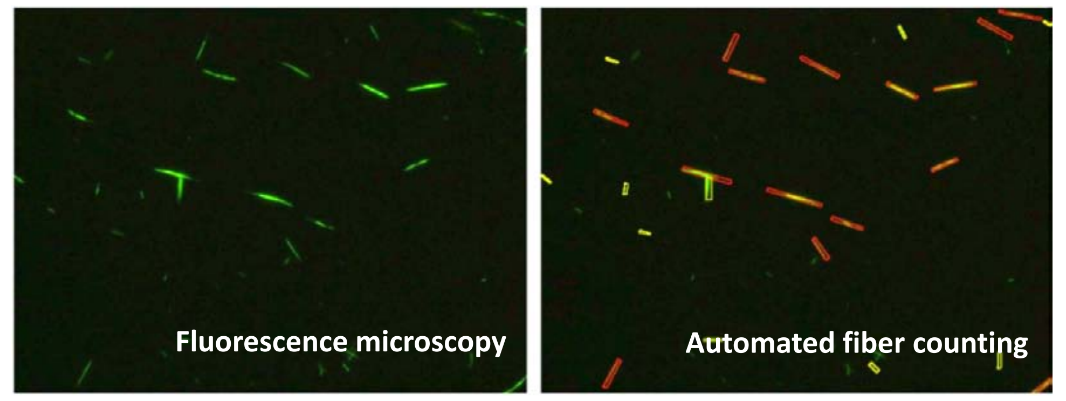
In collaboration with Hiroshima University, Siliconbio® has developed an airborne asbestos detection kit (**Asbester AIR**), using a fluorescently labeled DksA or GatZ protein probes that selectively bind to asbestos fibers. Following airborne dust sampling, just a few drops of reagents are applied onto a membrane filter. Immediate sample observation and high contrast imaging are possible without any complicated sample preparation.

On-site asbestos detection

Primo Star iLED (Carl Zeiss) is a portable LED fluorescence microscope that could be used for on-site detection of airborne asbestos.

Automated fiber counting

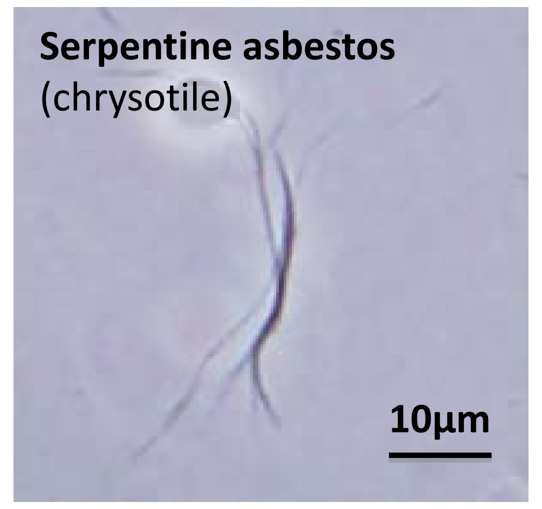
INTEC

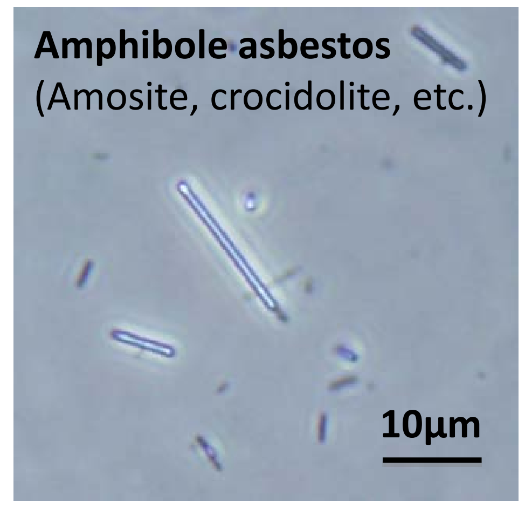


At present, we are developing of an FM-based automated asbestos detection and counting system. **This system would enable speedy fiber counting while considerably reducing inter-operator and inter-laboratory variability in asbestos counts.** Using the image analysis software developed by INTEC Inc., we could automatically detect fibers with aspect ratio >3 and length > 5 µm on FM images (see the picture to the left). The software requires only a few seconds to complete fiber counting.

Introduction

Asbestos


Serpentine asbestos (chrysotile)

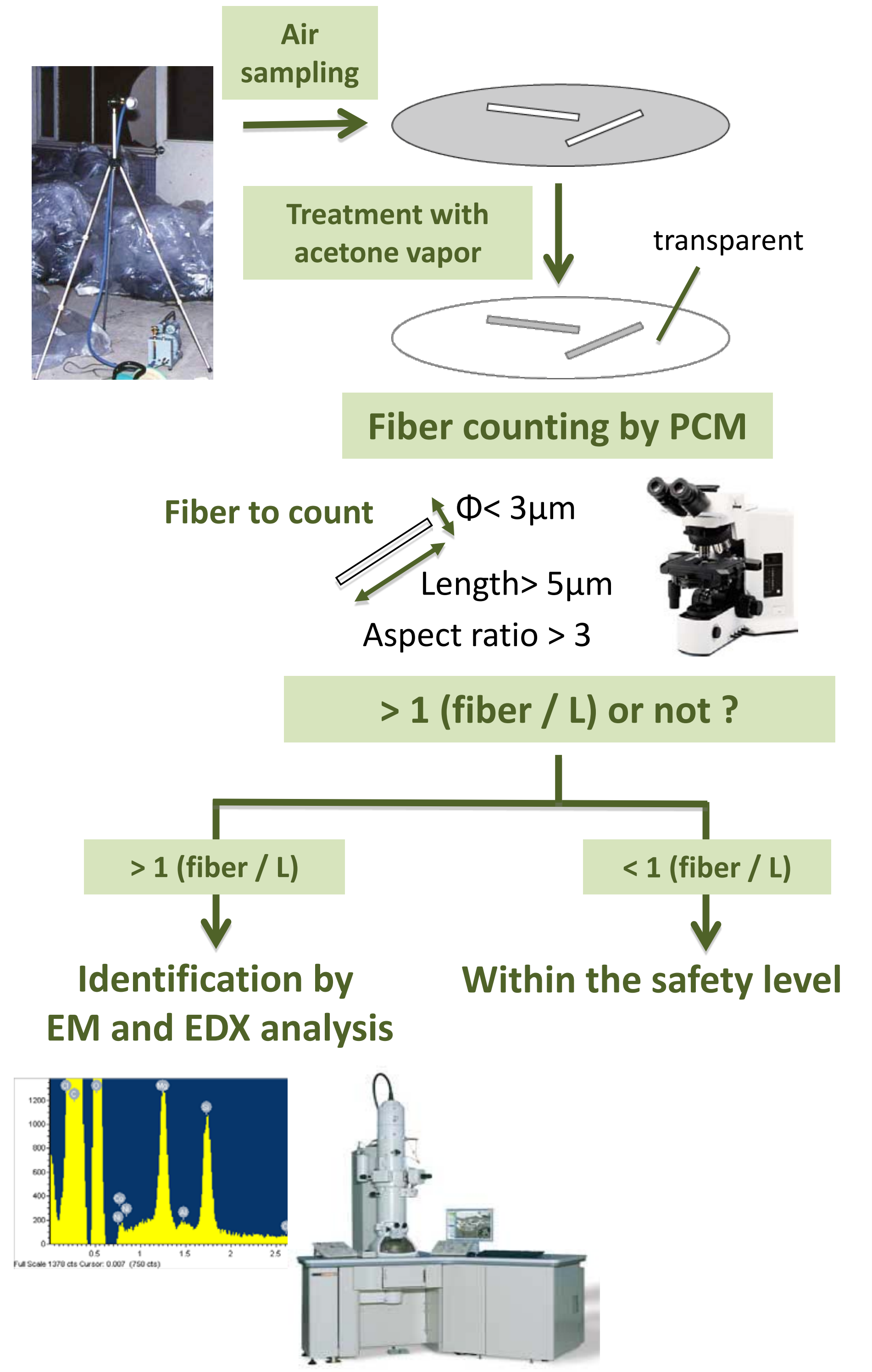

Amphibole asbestos (Amosite, crocidolite, etc.)

In Japan, demolition of asbestos-containing buildings is expected to peak between 2010 and 2035, posing considerable risks of asbestos exposure. **It is therefore necessary to develop a speedy on-site asbestos detection method.**

Currently used detection method for airborne asbestos fibers involves filtering air through a membrane filter with an air pump; the membrane is then rendered transparent with acetone vapor, and is examined using phase-contrast microscope (PCM). This method cannot distinguish asbestos from non-asbestos fibers. According to Japanese regulations, if fiber concentration is over one fiber per liter, we should perform further analysis using electron microscopy (EM). EM is not only able to detect the thinnest fibers, but could also be used for elemental analysis of each fiber by EDX, making it a complete fiber identification and counting tool. However, EM analysis requires expensive equipment and specialized skills, and is time-consuming.

Recently, we developed fluorescently labeled DksA and GatZ protein probes that selectively bind to asbestos fibers, making it possible to use fluorescence microscopy (FM) for asbestos detection. **With a portable fluorescence microscope, our method could be used for on-site monitoring of airborne asbestos, for example during demolition work.**

Asbestos monitoring manual ver. 4.0
(Japanese Ministry of the Environment)



Air sampling

Treatment with acetone vapor

transparent

Fiber counting by PCM

Fiber to count
Φ< 3µm
Length> 5µm
Aspect ratio > 3

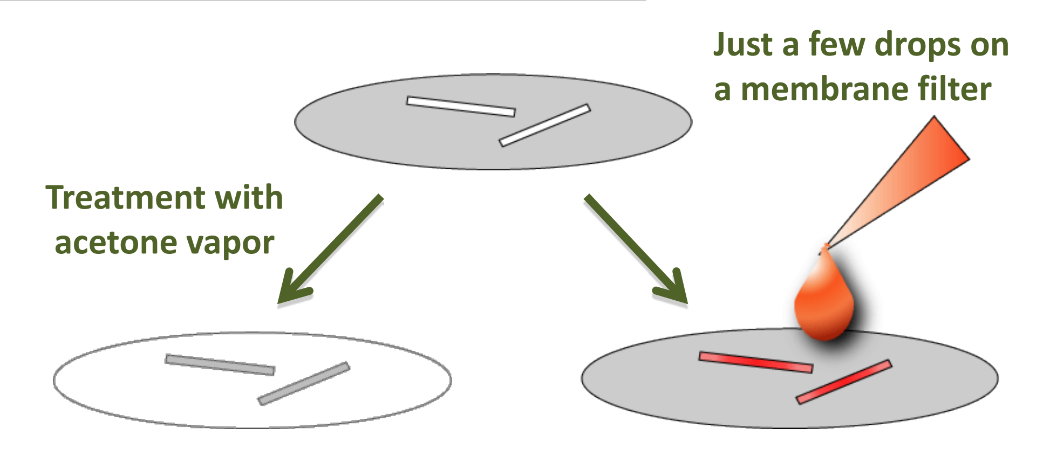
> 1 (fiber / L) or not ?

> 1 (fiber / L) → **Identification by EM and EDX analysis**

< 1 (fiber / L) → **Within the safety level**

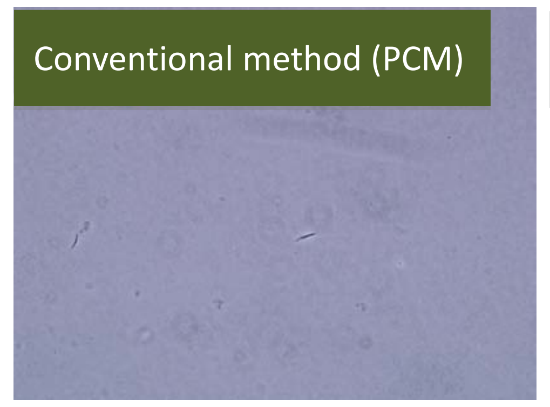
2. Fluorescence microscopy (FM)-based method

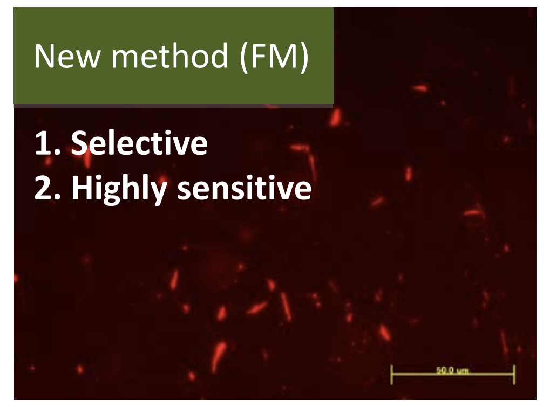
Methods (PCM and FM)



Treatment with acetone vapor

Just a few drops on a membrane filter


Conventional method (PCM)


New method (FM)

1. Selective
2. Highly sensitive

Sensitivity

To compare the number of fibers that could be detected by FM and PCM, we analyzed the wedges of the same filter sample using both methods.

Chrysotile Filter No.	FM (DksA-Cy3) Number of a) Graticule fields	FM (DksA-Cy3) Number of b) Fibers	PCM Number of a) Graticule fields	PCM Number of b) Fibers	FM/PCM relative fiber counts ※
1	50 50	149 178	50 50	30 15	7.3
2	42 50	201 200	50 50	45 37	5.3

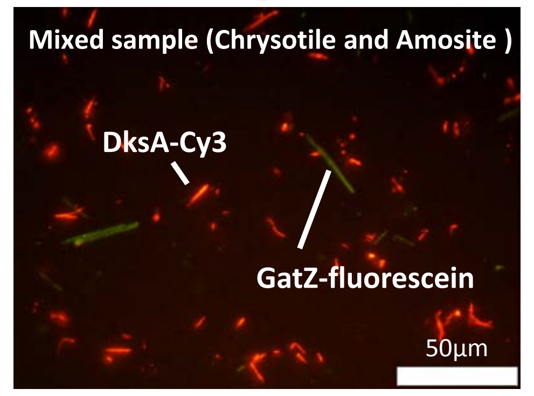
Amosite Filter No.	FM (GatZ-fluorescein) Number of a) Graticule fields	FM (GatZ-fluorescein) Number of b) Fibers	PCM Number of a) Graticule fields	PCM Number of b) Fibers	FM/PCM relative fiber counts ※
1	40 37	202 205	37 35	202 202	0.96
2	37 29	205 203	35 30	202 203	0.99

Crocidolite Filter No.	FM (GatZ-fluorescein) Number of a) Graticule fields	FM (GatZ-fluorescein) Number of b) Fibers	PCM Number of a) Graticule fields	PCM Number of b) Fibers	FM/PCM relative fiber counts ※
1	30 29	205.5 202	29 30	206 204	0.99
2	29 30	201.5 210	30 31	200.5 205	1.05

$$※\text{Relative fiber count (FM/PCM)} = \frac{(\text{Number of fibers / Number of graticule fields})_{\text{FM, two wedges}}}{(\text{Number of fibers / Number of graticule fields})_{\text{PCM, two wedges}}}$$

Selectivity

It is possible to distinguish chrysotile and amphibole asbestos.



It is possible to distinguish asbestos fibers from man-made mineral fibers (MMMF).

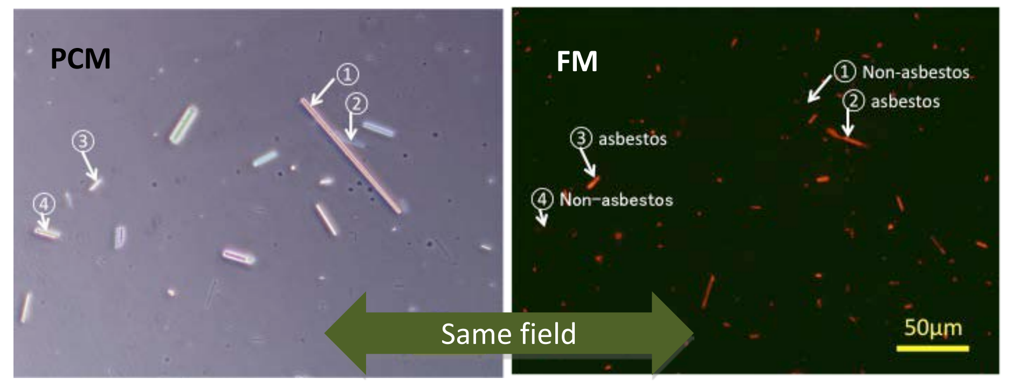


Table: Binding specificity of DksA and GatZ

Asbestos & MMMF	DksA	GatZ
Chrysotile	+	+/-
Crocidolite	-	+
Amosite	-	+
Anthophylite	-	+
Actinolite	-	+
Tremolite	-	+
Glass Wool	-	-
Rock Wool	-	-

Conclusion

	Electron microscopy (EM)	Fluorescence microscopy (FM)	Phase contrast microscopy (PCM)
Sensitivity	High	High	Low (> 0.25µm)
Selectivity	○ (high)	○	X
Measurement time	10h (1000 fields)	1h (50 fields)	1h (50 fields)
Portability	X	○	○
Automated fiber counting	X	○	X

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